Investigating the use of impaction samplers and qPCR methods for detection of foliar pathogens in potato fields, 2023

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Late blight detections were reported in Montcalm County, Michigan in 2022 and in neighboring regions in 2019, 2020, and in 2021. Spore samplers coupled with quantitative PCR assays have the potential to be an efficient and inexpensive tool for early detection of late blight outbreaks. Rotating arm impaction samplers were built and deployed during the 2023 growing season to: 1) monitor for *P. infestans* sporangia in commercial fields 2) investigate the range of detection from an inoculated field, and 3) compare sporangia detection levels to those of a suction-based spore sampler.

Materials and Methods

i. Commercial detection

Twelve rotating arm impaction samplers were built based on schematics developed by the MSU Small Fruit, Field Crops, and Forest Pathology programs (Fig. 1). Samplers were placed near six commercial potato fields located in Montcalm and St. Joseph counties. One sampler was deployed at each commercial site and at the Montcalm Research Center in Stanton, MI. The Montcalm fields were selected for proximity to previous late blight detections in 2022. Each week, sampling rods were collected and transported to MSU where their DNA was extracted, and a quantitative polymerase chain reaction (qPCR) assay was performed (Lees et al. 2012).

Fig. 1. Rotating arm impaction sampler deployed in a commercial potato field in 2023.

ii. Range from an inoculated source

The range of detection for these spore samplers was evaluated using an inoculated potato research trial located at the Plant Pathology Farm in East Lansing, MI. The field was inoculated on August 31 and symptoms were first detected visually on September 7. One sampler was placed on the Eastern edge of the field and served as a positive control. Two more were placed 500 m northwest of the inoculated field, a distance which has previously been used as an outer limit of spore sampler detection (Aylor et al. 2011). One trap was maintained at a height of 4 ft, the other was positioned at 20 ft to better capture long-distance sporangial movement. All three samplers were monitored weekly.

iii. Spore sampler comparison

The rotating arm samplers were also compared with a Burkard 7-day recording volumetric spore trap, which has been shown to collect higher concentrations of spores and was therefore considered a ‘gold’ standard (Sutton and Jones 1976; Aylor 1993). One rotating arm sampler was placed at the East edge of the inoculated late blight field, immediately adjacent to a Burkard sampler. Rods from the rotating arm sampler were collected daily, and the Cq values were used to estimate the average number of sporangia/ml air (Aylor 2017). For the Burkard samplers, sections of tape corresponding to one day of sampling were examined under a compound light microscope at 100x magnification. Putative sporangia were counted on two cross-sections of the tape comprising one third of the sampling area and counts were normalized to sporangia/ml air.

Results and Conclusions

i. Commercial detection

No late blight was reported in MI in 2023, which was consistent with qPCR results as no sporangia were detected from samplers placed in commercial fields. Preliminary tests verified that the qPCR assay was sensitive to a single sporangium, even on rods coated with grease and field soil, to mimic realistic samples. Standard curves were produced correlating Cq values to concentrations of DNA and numbers of sporangia extracted from the rods (Fig. 2). Future optimization of this assay will implement a more inhibitor-tolerant master mix to improve qPCR efficiency.

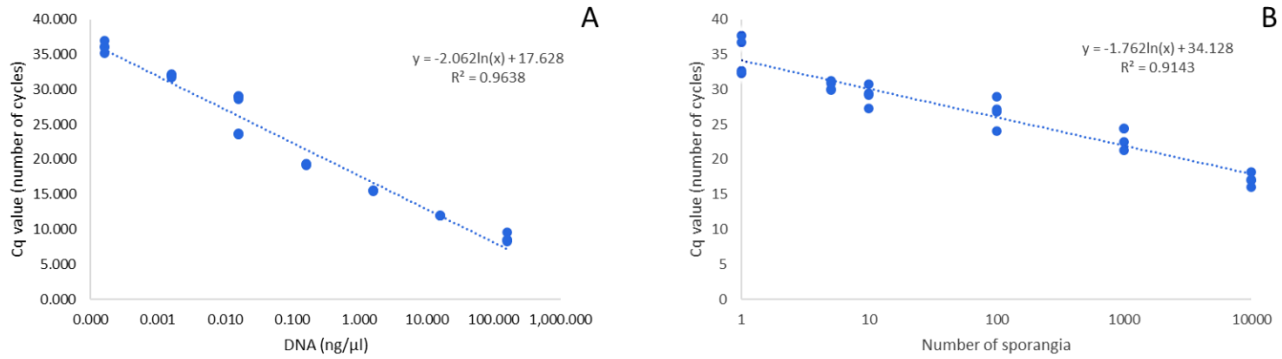

Fig. 2. qPCR standard curves generated using (A) serial dilutions for *P. infestans* DNA and (B) DNA extracted from rods inoculated with known quantities of *P. infestans* sporangia (Thiessen et al 2016).

ii. Range from an inoculated source

The sampler placed on the edge of the inoculated field detected *P. infestans* on the same week as visual scouting. However, no positive detections were made 500 m away at 4 ft and the 20 ft sampler only detected *P. infestans* once during peak infection (Table 1).

Table 1. Cq values obtained from samplers near an inoculated late blight field at the plant pathology farm in East Lansing, MI. Samples were collected weekly, and dates represent the last date of the sampling period. Dates marked with a "-" had no detectable level of late blight in the qPCR test. Dates marked with an "x" were not sampled at that location. The field was inoculated on Aug 31 and the first visual detection was made on Sep 7.

Location	Sampler	July					August				September				October				
	Height (ft)	3	10	17	25	31	7	14	21	28	5	11	18	25	2	9	16	23	30
Field edge	4	-	-	-	-	-	-	**36.6**	-	-	-	**34.7**	**28.6**	**38.3**	**33.2**	**37.5**	-	**29.2**	-
500m NE	4	x	x	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-
500m NE	20	x	x	x	x	x	-	-	-	-	-	-	**32.7**	-	-	-	-	-	-

iii. Spore sampler comparison

The Burkard was able to detect infection three days before visual late blight, while the rotating arm sampler made its first detection four days after visual detection. As expected, the Burkard sampler consistently detected higher levels of sporangia compared to the rotating arm sampler (Table 2). Overall, the rotating arm samplers were less sensitive than Burkard standard; however, they offer potential sensitive and low-cost options capable of detecting sporangia within a week of visual symptoms, providing useful information to growers.

Table 2. Comparison of daily estimates of airborne sporangia/ml air detected by the Burkard and rotating arm samplers. Bolded values indicate positive sporangia detections.

Date	Burkard (sporangia/ml air)	Rotating Arm (sporangia/ml air)	Date	Burkard (sporangia/ml air)	Rotating Arm (sporangia/ml air)
8/31/2023[a]	0.00	0.00	9/16/2023	**0.95**	0.00
9/1/2023	0.00	0.00	9/17/2023	**2.32**	0.00
9/2/2023	0.00	0.00	9/18/2023	**2.00**	**0.01**
9/3/2023	0.00	0.00	9/19/2023	**3.79**	**0.02**
9/4/2023	**0.32**	0.00	9/20/2023	**8.75**	**0.05**
9/5/2023	**0.11**	0.00	9/21/2023	**12.86**	0.00
9/6/2023	**0.11**	0.00	9/22/2023	**24.45**	0.00
9/7/2023[b]	**0.21**	0.00	9/23/2023	**50.89**	**0.04**
9/8/2023	**0.21**	0.00	9/24/2023	**182.40**	0.00
9/9/2023	**0.11**	0.00	9/25/2023	**295.99**	0.00
9/10/2023	**0.32**	0.00	9/26/2023	**161.01**	0.00
9/11/2023	**0.53**	**0.01**	9/27/2023	**359.53**	0.00
9/12/2023	**0.11**	0.00	9/28/2023	**89.88**	0.00
9/13/2023	**0.11**	0.00	9/29/2023	**34.56**	**0.02**
9/14/2023	**0.11**	**0.01**	9/30/2023	**21.92**	**0.01**
9/15/2023	**0.21**	**0.02**	10/1/2023	**19.81**	**0.18**

[a] Date the field inoculation
[b] Date of first visual late blight detection

Overall Summary

First-year testing demonstrated that the rotating arm impaction samplers, combined with qPCR assays, can be used detect late blight from fields in Michigan and may be of useful to augment visual scouting efforts. Additional optimization is needed to address limitations in range of detection and efficiency before large-scale implementation of spore samplers becomes a viable option for commercial growers. Experiments will be repeated in 2024.

Acknowledgements

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